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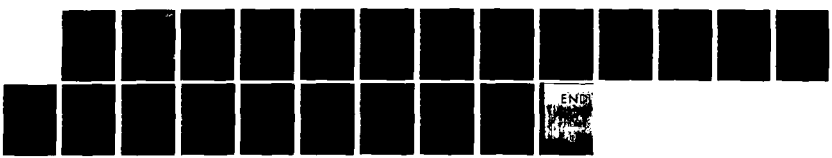

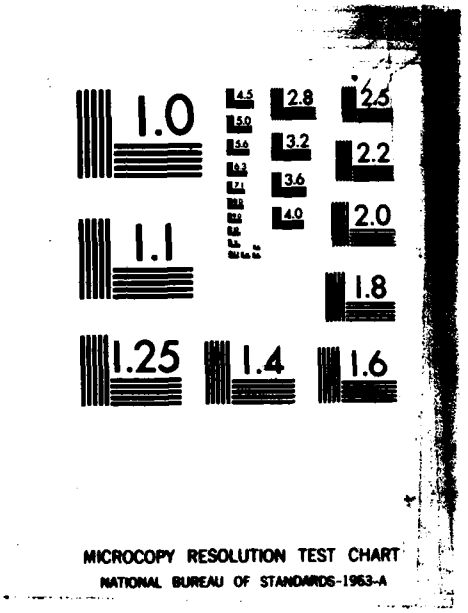
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IN VIVO SCREENING OF RADIOPROTECTORS

ANNUAL SUMMARY REPORT

JACOB J. CLEMENT, Ph.D.

JULY 1981

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract DAMD17-80-C-0143

Arthur D. Little, Inc.
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The USAMRDC maintains an active antiradiation drug development program to find and to evaluate chemoprophylactic agents capable of protecting military field personnel from the radiation effects of nuclear weapons. The purpose of the work described in this report is to establish an in vivo screening capability for the USAMRDC and to begin evaluating the ability of submitted compounds to protect normal body tissues from radiation injury.

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Experimental animals were chosen on the basis of general health, freedom from specific pathogens, radiation response, and supplier capability. Three mouse strains were considered and mice from five suppliers were evaluated. Female C57Bl/6 mice from the Charles River Breeding Laboratories were chosen. Radiation doses which cause death to 50% and 99% of mice from bone-marrow radiation injury were 809 rads and 971 rads, respectively. Gastrointestinal radiation lethality was caused in 50% of treated mice by a radiation dose of 1176 rads ($LD_{50/6}$).

Twenty-five compounds were received for radioprotectant evaluation. Eight of these agents, known radioprotectors, underwent detailed drug toxicity testing prior to being entered into radioprotector testing protocols. Drug toxicity data were also used to establish maximally tolerated nontoxic doses to be used in radioprotector studies. Seventeen newly synthetized compounds are currently being evaluated as antiradiation drugs. This testing is in progress and will be completed by the end of the current contract year.

SUMMARY

The USAMRDC maintains an active antiradiation drug development program to find and to evaluate chemoprophylactic agents capable of protecting military field personnel from the radiation effects of nuclear weapons. The purpose of the work described in this report is to establish an in vivo screening capability for the USAMRDC and to begin evaluating the ability of submitted compounds to protect normal body tissues from radiation injury. Experimental animals were chosen on the basis of general health, freedom from specific pathogens, appropriate radiation response, and supplier capability. Three mouse strains were considered, and mice of one strain from five suppliers were evaluated. B57B1/6 mice previously exposed to Sendai-virus and Sendai-free mice were tested. Mice from several suppliers were found to be in poor health, failed to gain weight in quarantine, and in some cases were found by a veterinarian pathologist to bear *Pseudomonas, Klebsiella,* or *Pasteurella.* Female C57B1/6 mice, Sendai-exposed, from the Charles River Breeding Laboratories were selected as an appropriate test animal. Radiation characterization studies indicated that doses of 809 rads and 971 rads killed 50% and 90% of these mice by bone-marrow toxicity . Gastrointestinal death was caused within six days to 50% of exposed mice by 1176 rads.

Eight compounds were submitted for detailed drug toxicity testing. Toxicity testing is complete for six of these agents given orally or intraperitoneally. Maximally tolerated drug doses have been established for seven of the compounds, and these are being evaluated for radioprotectant activity. Seventeen newly synthesized drugs have also been received as priority 1 compounds for radioprotector evaluation. Testing of these agents is in progress and will be completed by the end of the current contract year.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

Modern nuclear weapons are capable of producing medical illness with varying degrees of severity and from minutes to months after exposure. The manifestations of radiation sickness are dependent upon the dose of radiation received. Following relatively low doses of 100 to 300 rads, symptoms such as malaise, fever, and fatique are exhibited. These signs, the result of bone-marrow atrophy, infection, hemorrhage, and anemia usually appear two-three weeks after exposure. Exposure to a moderate radiation dose of 500 to 1000 rads results in nausea, vomiting, diarrhea, electrolyte loss, and circulatory collapse which usually begins in three to five days. Radiation doses of 2000 rads and above cause convulsions, tremors, ataxia, and loss of consciousness from minutes to hours after exposure. All three radiation syndromes result in mortality with survival possible only at doses below about 500 rads.

Drug protection against nuclear radiation injury has been recognized as a militarily significant method to decrease combat casualties and to increase personnel performance by decreasing both the onset and severity of radiation illness. Both the U.S. Air Force Radiation Laboratory and the U.S. Army Medical Research and Development Command (USAMRDC) have tested antiradiation drugs. The Air Force program has tested over 1500 compounds, and the USAMRDC program has tested over 4,400 compounds. Only about two hundred of these compounds have shown activity in mice; and of these compounds, relatively few appear potentially useful in humans. To be of military use, an antiradiation drug should protect personnel when taken orally, be effective for several hours, and have no serious side effects. The degree of protection is also important. While total radiation protection is desirable, a reduction by a factor of 2-3 in the effective radiation dose appears to be a reasonable expectation and an attainable goal. Towards this end, the USAMRDC sponsors a rational drug development program based on established structure-activity relationships as well as on promising new directions. The purpose of this contract, "In Vivo Screening of Radioprotectors," is to evaluate the effectiveness of newly synthesized chemoprophylactic agents and to characterize in detail promising agents which have shown protectant activity in an in vivo screen. Data presented in this report represent the results of our screening effort during the first year of this contract. The studies described include (a) selection and characterization of experimental animals, (b) drug toxicity studies of known protectants, and (c) description of radioprotectant screening studies.

MATERIALS AND METHODS

Experimental Animals

Female mice were used in all cases. C57B1/6, CDF_1, and BDF_1 strains were obtained from various vendors as presented in the Results section. Mice were supplied as Sendai virus-free and as having a positive Sendai titer. Selected groups of mice were also cultured for bacterial infection by Dr. James Fox of the Massachusetts Institute of Technology who is a consultant to Arthur D. Little, Inc., in the capacity of veterinarian pathologist.

No anesthetic was used in these studies. Mice surviving 30 days after drug or radiation treatment were euthanized in a CO_2 chamber. During quarantine and post-treatment holding, mice were kept on a 12-hour light cycle and allowed laboratory chow and hyperacidified or hyperchlorinated water *ad libitum*.

Experimental Drugs

Compounds were supplied by the COTR, Colonel David Davidson of the Walter Reed Army Institute of Research. Samples were stored frozen and shielded from light. Prior to use, samples were slowly warmed to room temperature. Chemicals were dissolved in water when soluble or suspended in hydroxypropylcellulose (Klucel) when insoluble. Drug doses used in this report are corrected for salt and water content. Compounds were prepared immediately prior to use and injected by individual animal body weight.

Toxicity Testing

Eight of the 25 compounds received for testing were known to have radioprotectant activity and were of sufficient interest to the USAMRDC to warrant full drug toxicity evaluation. On initial dose finding, studies of each drug given intraperitoneally (ip) or orally (po) were performed using four mice per group. A detailed drug toxicity evaluation of each drug given ip or po was then designed based on the results of dose-finding studies. Drug doses which kill 50% (LD_{50}) and 10% (LD_{10}) of mice and the slope of the dose-survival curve were calculated by the Cornfield-Mantel modification of Karber's method (*J Am Stat Assoc* 45:181, 1950).

Irradiation Procedures

The irradiation unit used to expose mice is a 4,000 Curie J. L. Shepherd, Mark 1, Model #68 ^{137}Cs-irradiation unit (Serial #647) located in the Radiation Therapy Laboratory. Unanesthetized mice were placed in individual compartments of a circular plexiglass mouse holder which was rotated on a turntable during exposure. Eight to ten mice at a time were irradiated at a dose rate of approximately 300 rads/min.

RESULTS AND DISCUSSION

Selection and Characterization of Mouse Strain

The selection of a standard mouse for testing of antiradiation drugs is critically important to a screening effort. Obviously, a standard sex, age, and strain of mouse must be used. Mouse radiosensitivity is also quite dependent on the animals' general health as well as on the presence of particular bacteria and viri. For these reasons considerable emphasis must also be placed on the selection of a suitable animal supplier.

Female mice were used in this project since the reference protectant, WR-2721, is less toxic in female mice and because female mice tent to be less belligerent which minimizes nonradiation animal deaths. The three mouse strains evaluated were B57B1/6, CDF_1, and BDF_1 mice. The radiation sensitivity of these three mouse strains is shown in Table 1. Female mice, approximately 11 weeks old were supplied by either Charles River Breeding Laboratories (CRL) or Simonsen Laboratories (SIM) as noted. C57B1/6 mice and CDF_1 mice were very similar in radiosensitivity to both GI tissue ($LD_{50}/_6$ and $LD_{50}/_7$) and bone marrow ($LD_{50}/_{30}$ and $LD_{99}/_{30}$). BDF_1 mice appeared to be more sensitive than the other two strains. All three mouse strains exhibit good separation between the two acute radiation syndromes (Table 2) in that bone marrow toxicity (mortality after day 7) in 90% or more of exposed animals could be achieved with 10% toxicities or less attributable to GI damage (deaths prior to day 7).

C57B1/6 mice were chosen as the test strain after discussion with the COTR. This strain has been used by other investigators in the radioprotector field providing a common ground for comparison of data for published protectors with data for newly developed compounds. Due to economic considerations mice were originally provided by various vendors through a contract with The Mammalian Genetics and Animal Production Section, National Cancer Institute. Early mortality in a significant number of irradiated mice suggested possible bacterial infection, however. Selected mice were evaluated by Dr. James G. Fox a veterinarian pathologist from the Laboratory Animal Medicine Diagnostic Laboratory at the Massachusetts institute of Technology. Mice from some suppliers were found to be, in general, in poor health and failed to gain weight after several weeks in quarantine. Still other animals were found to have large titers for Sendai virus. Mice are supplied by vendors as Sendai-naive or as Sendai-positive. Exposure of Sendai-naive mice to the virus leads to a massive virus infection which may seriously affect the radiation response of the mice. Both Sendai-naive and Sendai-positive mice were evaluated. Data in Table 3 indicate that Sendai-naive animals can be maintained at our animal facility without becoming infected with the virus. Pathological testing of mice in newly arrive mice (8 and 9 weeks old) and mice in quarantine for three weeks (11 and 12 weeks old) showed no evidence of Sendai.

The radiation response of C57B1/6 mice positive for Sendai was evaluated in three separate studies (Table 4). Full radiation-dose survival studies were performed in two separate experiments. The bone-

Table 1

RADIATION RESPONSE OF THREE MOUSE STRAINS

	C57B1/6 (CRL)	CDF_1 (CRL)	BDF_1 (SIM)
$LD_{50/6}$	1176 rads	1127 rads	944 rads
95% CL	1132-1222	1102-1152	910-980
Probit Slope	23.1	53.5	19.1
$LD_{50/7}$	1049 rads	1095 rads	859 rads
95% CL	1008-1091	1067-1123	830-888
Probit Slope	19.7	32.2	20.4
$LD_{50/30}$	809 rads	769 rads	713 rads
95% CL	779-840	749-792	706-718
Probit Slope	29.3	26.9	59.6
$LD_{99/30}$	971 rads	939 rads	780 rads

Table 2

MORTALITY FREQUENCY IN THREE MOUSE STRAINS

Radiation Dose (rads)	No. of Mice	Deaths Prior to Day 7 (%)	Deaths After Day 7 (%)	Total 30-Day Mortality (%)
C57B1/6 (CRL)				
1329	16	100	0	100
1176	16	25	75	100
1041	16	25	75	100
921	16	0	94	94
815	16	0	56	56
CDF_1 (CRL)				
1331	20	100	0	100
1210	20	95	5	100
1100	20	30	70	100
1000	20	0	100	100
909	20	0	95	95
826	20	0	90	90
751	19	0	26	26
BDF_1 (SIM)				
1210	20	100	0	100
1100	20	80	20	100
1000	20	70	30	100
909	20	50	50	100
826	20	10	90	100
751	20	0	90	90

Table 3

MORTALITY OF SENDAI-NAIVE C57B1/6 MICE FOLLOWING

EXPOSURE TO 1000 RADS

Supplier	Age	No. of Mice	Deaths Prior to Day 7	Deaths After Day 7	Total Deaths
JAX	8 wks	10	0	10	10
JAX	11 wks	10	0	10	10
CRL	9 wks	8	0	8	8
CRL	12 wks	9	2	7	9

Table 4

RADIATION RESPONSE OF C57BL/6 FEMALE MICE

	Study 1	Study 2	Study 3
Mice/group	20	20	16
Supplier	LSC		CRL
Age	10 Wks		11 wks
$LD_{50/6}$	959 rads		1176 rads
95% CL	918-1002		1132-1222
Probit Slope	20.0		23.1
$LD_{50/7}$	907 rads		1049 rads
95% CL	865-950		1008-1091
Probit Slope	16.7		19.7
$LD_{50/30}$	701 rads	748 rads	809 rads
95% CL	688-713	735-762	799-840
Probit Slope	20.2	49.5	29.3
$LD_{99/30}$	914 rads	835 rads	971 rads

marrow radiation response only was characterized in another study. Although similar in some respects, substantial differences among $LD_{50/6}$ and $LD_{99/30}$ values were noted. These variations were probably due to differences in the health of animals between suppliers. Mortality frequency for these three studies are shown in Table 5. Charles River Breeding Laboratories was finally chosen as a supplier of C57Bl/6 mice due to availability of adequate numbers of animals, a consistently good reputation for good animal husbandry, and the ability to provide Sendai-positive mice. The last factor will enable researchers at other facilities which are not totally Sendai-free to use the same test animal.

Data in Tables 4 and 5 as well as published data from other investigators indicate that a radiation dose which will just kill 100% of treated C57Bl/6 mice is between 950 and 1000 rads. This has been confirmed in separate studies which also examine the effect of mouse age on the death patterns of these mice (Table 6). Radiation doses of 950 or 1000 rads consistently kills 100% of exposed mice. Younger mice (9 weeks) tend to die earlier with some indication of possible GI lethality. Twelve-week-old mice exposed to 1000 rads die between days 9 and 16 with no early death to insure consistent 100% lethality. On the basis of these data, the primary screening system for radioprotectants was chosen to be a whole-body dose of 1000 rads to 12-week-old female Sendai-positi C57Bl/6 mice from Charles River.

Drug Toxicity Determinations

Eight of the 25 compounds submitted by USAMRDC have previously been shown to have radioprotectant activity and were of sufficient interest to warrant a detailed determination of drug toxicity in mice. These compounds are described in Table 7. All eight compounds were entered in drug-dose range-finding studies. Six compounds were fully evaluated for drug toxicity and two compounds were found to be nontoxic at the highest drug doses tested. These two compounds were subsequently retested to confirm lack of toxicity and one compound was found to be nontoxic when administered intraperitoneally (ip) or orally (po).

A summary of the drug toxicity data for these 8 compounds is shown in Table 8. The toxicity data for the most part are consistent with available data on these compounds. Results of testing WR-2721 ip were inconclusive since the highest dose tested, 1200 mg/kg, was toxic to only 3 of 10 mice. Toxicity testing on this compound will be repeated at higher dose levels. The suggested MTD for WR-2721, 500 mg/kg, should remain unchanged, however, since this dose has been selected after discussion with the COTR as the preferred standard dose for this reference radioprotectant.

WR-77913 in a dose-finding study was found to be nontoxic following ip and po doses of 1734 mg/kg and 2600 mg/kg, respectively. WR-77913 was retested at these dose levels using 10 mice per group and was found to be nontoxic.

WR-151327 administered ip was not toxic to all mice at the highest dose tested and the pattern of mortality was not dose-responsive. WR-151327 administered po was initially found to be nontoxic at 2640 mg/kg. Subsequently tested at 2460 mg/kg and 1968 mg/kg using 10 mice per group,

Table 5

MORTALITY FREQUENCY IN C57BL/6 MICE

Radiation Dose (rads)	No. of Mice	Deaths Prior to Day 7 (%)	Deaths After Day 7 (%)	Total 30-Day Mortality (%)
Study 1				
1150	20	100	0	100
1000	20	50	50	100
870	20	30	70	100
756	20	0	85	85
658	20	0	5	5
572	20	0	10	10
497	20	0	5	5
Study 2				
870	20	75	25	100
811	20	45	55	100
756	20	5	50	55
705	20	0	5	5
658	20	0	5	5
Study 3				
1329	16	100	0	100
1176	16	25	75	100
1041	16	25	75	100
921	16	0	94	94
815	16	0	56	56

Table 6

EFFECT OF AGE ON THE RADIATION RESPONSE OF C57BL/6 (CRL) MICE

Group			Number of Mice Dead on Day														
Age (wks)	Radiation Dose (rads)	No. Per Group	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21-23
9	1000	10	1		1	4	1		2	1								
10	950	14								2	2	2			2	2		4
10	1000	10			3	4		1	1	1								
12	950	20							3	3	5	1	4	2		1	1	
12	1000	20					1	6	1	3	5	2	1	1				

Table 7

RADIOPROTECTANTS EVALUATED FOR DRUG TOXICITY

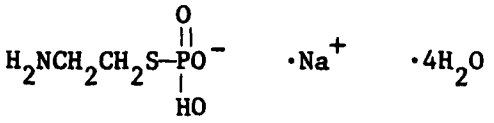
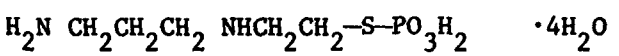
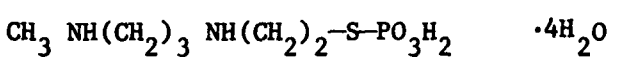
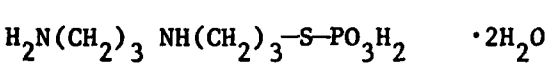
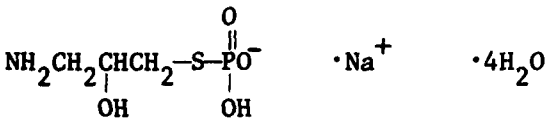
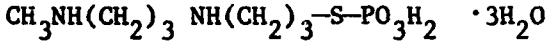
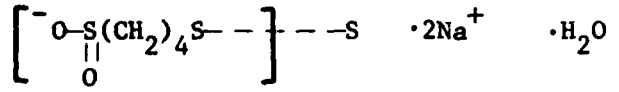
Compound	Batch No.	Chemical Structure
WR-638	BJ76356	
WR-2721	BJ45388	
WR-3689	BJ78538	
WR-44923	BJ40025	
WR-77913	BJ78529	
WR-151327	BJ40016	
WR-168643	BJ44774	
WR-176542	BJ44569	

Table 8

SUMMARY OF DRUG TOXICITY FOR RADIOPROTECTANTS

Compound	Bottle No.	Rte of Admin	LD_{50} (mg/kg)[a]	95% CL (mg/kg)	Probit Slope	LD_{10} (mg/kg)	Suggested MTD[b] (mg/kg)	Status
WR-638	BJ 76356	ip	1089	931-1273	4.3	547	500	Complete
		po	974	854-1111	7.4	653	600	Complete
WR-2721	BJ 45388	ip					500	Inconclusive
		po	985	930-1044	9.4	718	650	Complete
WR-3689	BJ 78538	ip	1242	1165-1325	8.4	873	800	Complete
		po	1689	1574-1812	8.2	1176	1000	Complete
WR-44923	BJ 40025	ip	432	385-486	8.3	303	250	Complete
		po	721	664-782	17.3	608	500	Complete
WR-77913	BJ 78529	ip				>1734	1500	Complete
		po				>2600	2000	Complete
WR-151327	BJ 40016	ip						Inconclusive
		po						Inconclusive
WR-168643	BJ 44774	ip	804	759-851	16.9	675	600	Complete
		po	1150	1093-1209	12.5	909	800	Complete
WR-176542	BJ 44569	ip	329	312-348	10.7	250	200	Complete
		po	757	725-790	17.0	637	550	Complete

[a]Drug doses corrected for salt and water content and expressed as free-base doses.

[b]MTD's are approximately 90% of LD_{10} values and rounded for convenience.

WR-151327 was toxic to 9/10 and 8/10 mice, respectively. Both initial dose finding and full toxicity studies will be repeated.

Based on drug toxicity data, suggested maximally tolerated doses (MTD) have been calculated as 90% of each compound's LD_{10} value and rounded for convenience. The suggested MTD's are being used as starting doses for bone marrow radioprotection studies.

Radioprotection Studies

Two types of compounds are being evaluated in bone-marrow radioprotection studies. Compounds are either established radioprotectants (8 compounds) or compounds submitted by the USAMRDC for initial radioprotection characterization (17 compounds). Established radioprotectants are being tested for effectiveness after ip and po administration. These drugs are administered ip at the MTD, 1/2 MTD, 1/4 MTD, and 1/8 MTD. Oral drug administration is at the MTD, 1/2 MTD, and 1/4 MTD. Ten mice per treatment group are used and all drugs except WR-168643 are administered 30 min prior to whole-body irradiation at 1000 rads. WR-168643 is administered 10-15 min prior to irradiation. Control groups consist of mice receiving no treatment, radiation alone, and 500 mg/kg WR-2721 plus radiation.

The 17 newly synthesized and untested compounds are available in limited amounts and are therefore being screened by another protocol. These agents are being evaluated after ip administration only at doses of 600, 300, and 150 mg/kg. Most agents are administered 30 min prior to 1000 rads. Analogs of WR-168643 are administered 15 min prior to irradiation. Control groups consist of mice receiving no treatment, radiation alone, and 500 mg/kg WR-2721 plus radiation. These studies are currently on test or scheduled for testing and completion prior to the end of the current contract year. Results are therefore not yet available.

CONCLUSIONS

The first year of this radioprotectant screening contract has been devoted to the selection of a suitable experimental animal, quantitative drug toxicity studies of 8 protectants of special interest to the USAMRDC, and initial bone-marrow protection studies of 25 compounds. These 25 compounds consist of 8 known protectants and 17 newly synthesized agents. Mice chosen as being the most suitable for this screening effort are 12-week-old female C57Bl/6 mice for the Charles River Breeding Laboratory and bearing a positive Sendai-virus blood titer. These animals have a suitable radiation response, are in good general health, and can be housed in most animal holding facilities without elaborate quarantine measures. Drug toxicity determinations of several protectants formed the basis for establishing maximally tolerated doses for use in bone-marrow protection studies. Protection from hematopoietic lethality is currently being established for 25 submitted compounds. The antiradiation drug screening program is, therefore, considered to be fully operational and capable of providing the USAMRDC with rapid, reliable evaluation of newly synthesized compounds as well as detailed characterization of compounds known to protect normal tissues from radiation damage.

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